Application Note

CD-0033

Evaluation of structural change and thermal stability of Bovine IgG

Introduction

Evaluating the structure and stability of protein is extremely important for utilization in biopharmaceutical and such industries. CD spectra in far-ultraviolet region reflects to the secondary structure of protein. Therefore CD spectra measurement in far-ultraviolet region is widely used for evaluation of protein structure and it's stability. As optical absorption derived from buffer solution in far-ultraviolet region is strong, it is necessary to contrive some preparative techniques so as to perform the measurement properly. In general, reducing buffer concentration and using a short path length cell are more effective to the measurement. PTC-510 water-cooled peltier cell holder has design side capability to fit with short path length cylindrical cell, which made it possible to measure CD spectrum of protein sample in buffer down to far-ultraviolet region. In this application note, CD spectra measurement of Bovine IgG / phosphate buffer solution was performed using Temperature Interval Scan Measurement Program and a short path length cylindrical cell. Measurement temperature was set from 20ºC to 95ºC.

Keyword: Structure change, Thermal stability, Short path length cell, Far-ultraviolet

Measurement condition

Measurement mode:	CD, Abs
Measurement range:	190 - 250 nm
Data interval:	0.5 nm
Scan speed:	200 nm/min
Response:	1 sec
Bandwidth:	1 nm
Accumulation:	2 times
Temperature range:	20 - 95ºC
Temperature rising:	1ºC/min
Data interval:	1ºC
Measurement time:	75 minutes*

* The temperature keep rising during spectrum measurement.

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Measurement Results

0.14 mg/mL Bovine IgG solution (50 mM phosphate buffer, pH6.9) was put into a cylindrical cell with an optical path length of 0.5 mm, then CD spectra was measured from 20ºC to 95ºC at 1ºC step (Figure1-a). The structural change of IgG were observed with temperature rising. In figure 1-b, temperature change of CD value and absorption at 216 nm were plotted. The thermal denaturation of IgG was observed at above around 60ºC. The increase of the absorbance was observed after the change of CD value, this is attributed to scattering of a measurement light caused by IgG aggregation.

1.1 mg/mL Bovine IgG solution (50 mM phosphate buffer, pH6.9) was put into a cylindrical cell with an optical path length of 0.1 mm, then CD spectra was measured from 20ºC to 95ºC at 1ºC step (Figure 2-a). The temperature change of CD value and absorption at 216 nm were plotted. Compared with low concentration solution (0.14 mg/mL), the absolute value of CD signal remarkably reduced by 80ºC, and reduction in absorbance was observed by 90ºC. This result indicates that, in the high concentration condition (1.1 mg/mL), absorption to a cell surface and precipitation of IgG are occurred due to the marked aggregation of IgG. So, the amount of IgG on optical axis of measurement light are reduced.

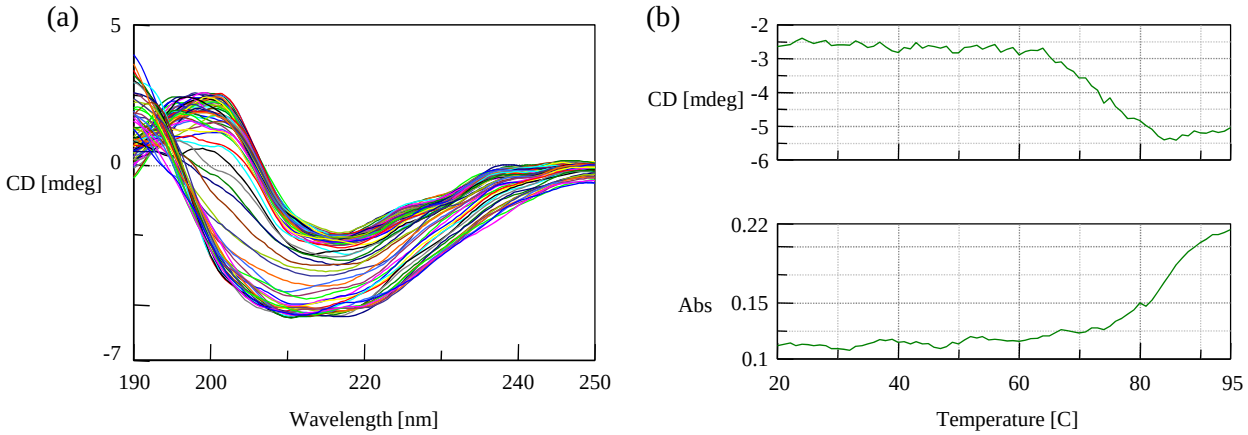

Fig. 1 The temperature change of CD spectra (a) and the change of CD and Abs at 216 nm (b) of low concentration Bovine IgG solution.
Concentration: 0.14 mg/mL, optical path length: 0.5 mm

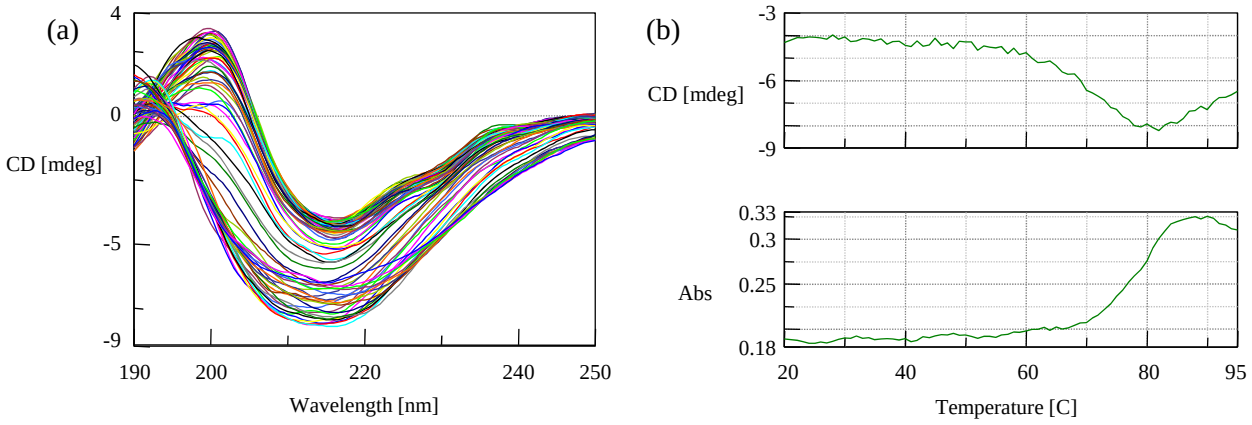

Fig. 2 The temperature change of CD spectra (a) and the change of CD and Abs at 216 nm (b) of high concentration Bovine IgG solution.
Concentration: 1.1 mg/mL, optical path length: 0.1 mm

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