Application Note
CD Spectroscopy

Microassay thermal denaturation of ribonuclease A probed by CD spectroscopy

Introduction

Circular dichroism (CD) is a useful technique to measure and analyze secondary structure and the thermal denaturation of proteins and nucleic acids in the far-UV region. Typically, a 1 mm pathlength rectangular cell is used for these measurements. However, this requires a sample volume of about 200 µL. In the case of samples where only very small volumes are available, JASCO now offers a new capillary cell for sample volumes smaller than 10 µL. The capillary cell also has a jacket for thermal ramping measurements, shown in Figure 1. CD measurements remain simple to perform and the capillary cells are inexpensive and disposable.

This application note demonstrates the use of a capillary cell and jacket for thermal ramping studies of ribonuclease A.

JASCO J-1500 CD spectrometer
View product information at www.jascoinc.com

Keywords

Circular dichroism, capillary cell, microassay, thermal denaturation, ribonuclease A, Denatured Protein Analysis software, denaturation temperature, J-1500

Experimental

Measurement conditions	
Data acquisition interval	0.2°C
Path length	0.5 mm (capillary cell), 1 mm (rectangular cell)
Spectral bandwidth	1 nm
Rising temperature rate	1°C/min
Measurement wavelength	222 nm
Response time	8 sec

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1 mg/mL of ribonuclease A aqueous solution is drawn up into a capillary cell with a 0.5 mm optical pathlength, as seen in Figure 1. The cell is then inserted into the capillary jacket for thermal ramping CD measurements.

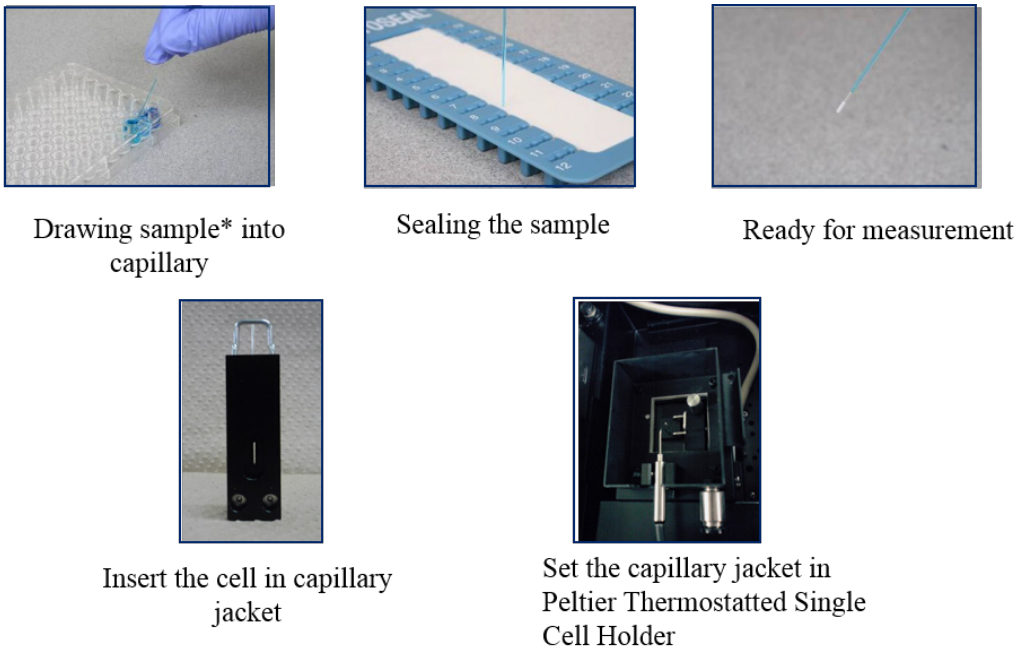

Figure 1. Capillary cell preparation diagram. For greater visibility of a sample in the photos above, a colored sample was used instead of ribonuclease A solution.

Results

Figure 1 shows the thermal denaturation of ribonuclease A. The JASCO JWTDA-519 Denatured Protein Analysis software was used to calculate the denaturation temperature of ribonuclease A in both the capillary and rectangular cells. 59.4°C was calculated for the capillary cell which is in good agreement with 59.7°C obtained for the denaturation temperature in the rectangular cell.

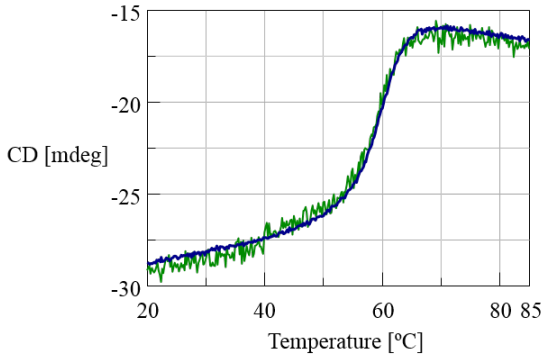

Figure 2. Thermal denaturation curve of ribonuclease A measured at a wavelength of 222 nm. The green curve was obtained using the capillary cell while the blue curve was measured using the 1 mm rectangular cell.

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Conclusion

This application note demonstrates that the denaturation temperature values, along with the thermal denaturation curves shown in Figure 2, illustrate that the microassay of ribonuclease A using the capillary cell and jacket can be carried out with high accuracy.

Note: The MSD-462 microsampling disc can be used for spectral scanning measurements on sample volumes between 2 and 10 µL. The MSD-462 applications are shown in the following Application Notes: 260-CD-0011 and 260-CD-0019.

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