Application Note

CD-0014

Thermal denaturation analysis of super thermostable cellulase

Introduction

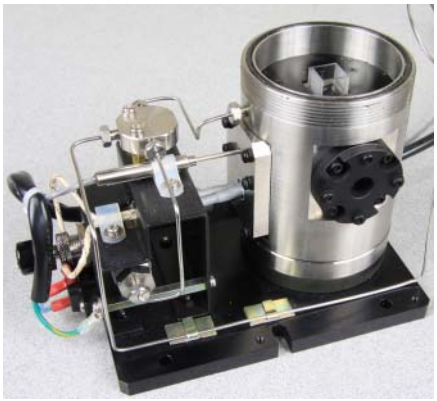
The thermodynamic properties and secondary structures of proteins reveal important information on their functions. Differential scanning calorimetry (DSC) and CD spectroscopy are usually used to measure the thermal denaturation of proteins. CD spectroscopy has advantages such that CD spectra can be measured using lower concentrations of proteins than DSC and can also be measured at various pHs and in a wider range of solvent conditions. However, CD spectra cannot be measured at the temperature above 100ºC because the boiling point of water is 100ºC.

JASCO has developed the TC-700/700PC type, high pressure-resistant/high temperature measurement sample chamber for high temperature measurements above 100ºC. TC-700 enables measurement of CD spectra at temperatures up to 170ºC by pressurizing the sample solution to 1 MPa using commercially available high-pressure N_2 gas. Using TC-700, the thermal denaturation of super thermostable proteins, such as those originating from thermophiles, can be measured.

Herein, thermal denaturation analysis of super thermostable cellulase using TC-700PC is explained. Super thermostable cellulase maintains its activity at temperatures up to 90ºC. Research on enzymes, such as super thermostable cellulase, may lead to the development of methods for the production of bioethanol from agricultural waste rather than from important crops, such as corn or sugarcane.

Keywords: Thermostable proteins, Thermal stability, Agricultural waste

<Specifications of TC-700/700PC>



TC-700 Sample chamber

Temperature Controller

Available cells:	Rectangular cells (1, 2, 5 and 10 mm) (1, 2 and 5 mm cells need corresponding cell spacers)
Temp. Control Region:	RT to 170ºC
Accuracy:	+/- 0.5ºC
Method:	PID control, 100 W heater Manual control (TC-700) PC control (TC-700PC)
Temp. Sensor:	Platinum temperature sensor

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<CD spectra measurement>

Measuring conditions

Conc.:	0.01 mg/mL	Pressure:	0.8 MPa	Temp.:	45, 80, 100 and 120ºC
Response:	2 sec	Sensitivity:	Standard	Wavelength:	260-195 nm
Data interval:	0.1 nm	Scan speed:	100 nm/min	Cell pathlength:	10 mm
Bandwidth:	1 nm				

1.2 mg/mL super thermostable cellulase in 20 mM Tris-HCl buffer (pH 8.0) was diluted with distilled water and measured.

Results

The CD spectra of super thermostable cellulase measured at 45, 80, 100 and 120ºC are shown in Fig. 1. The thermal denaturation is observed at temperature of only over 100ºC.

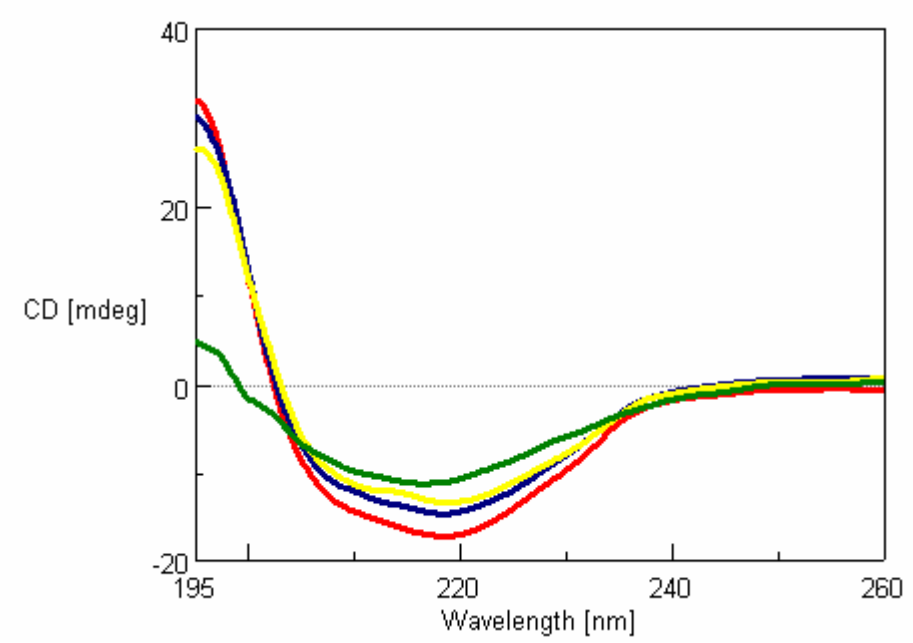

Fig. 1 CD spectra of super thermostable cellulase
Red line: 45ºC, Blue line: 80ºC, Yellow line: 100ºC, Green line: 120ºC

<CD spectra measurement>

Measuring conditions

Conc.:	0.025 mg/mL	Pressure:	0.8 MPa	Temp. range:	80-120ºC
Temp. interval:	0.1ºC	Temp. slope:	1ºC/min	Bandwidth:	1 nm
Cell pathlength:	10 mm	Wavelength:	220 nm		

Results

The thermal denaturation of super thermostable cellulase was monitored at 220 nm. The result of the analysis using the [Denaturation Analysis] program is shown in Fig. 2. There is a sharp decrease in the CD value at temperatures over 100ºC. The melting temperature (Tm) of super thermostable cellulase is shown to be 106.3ºC.

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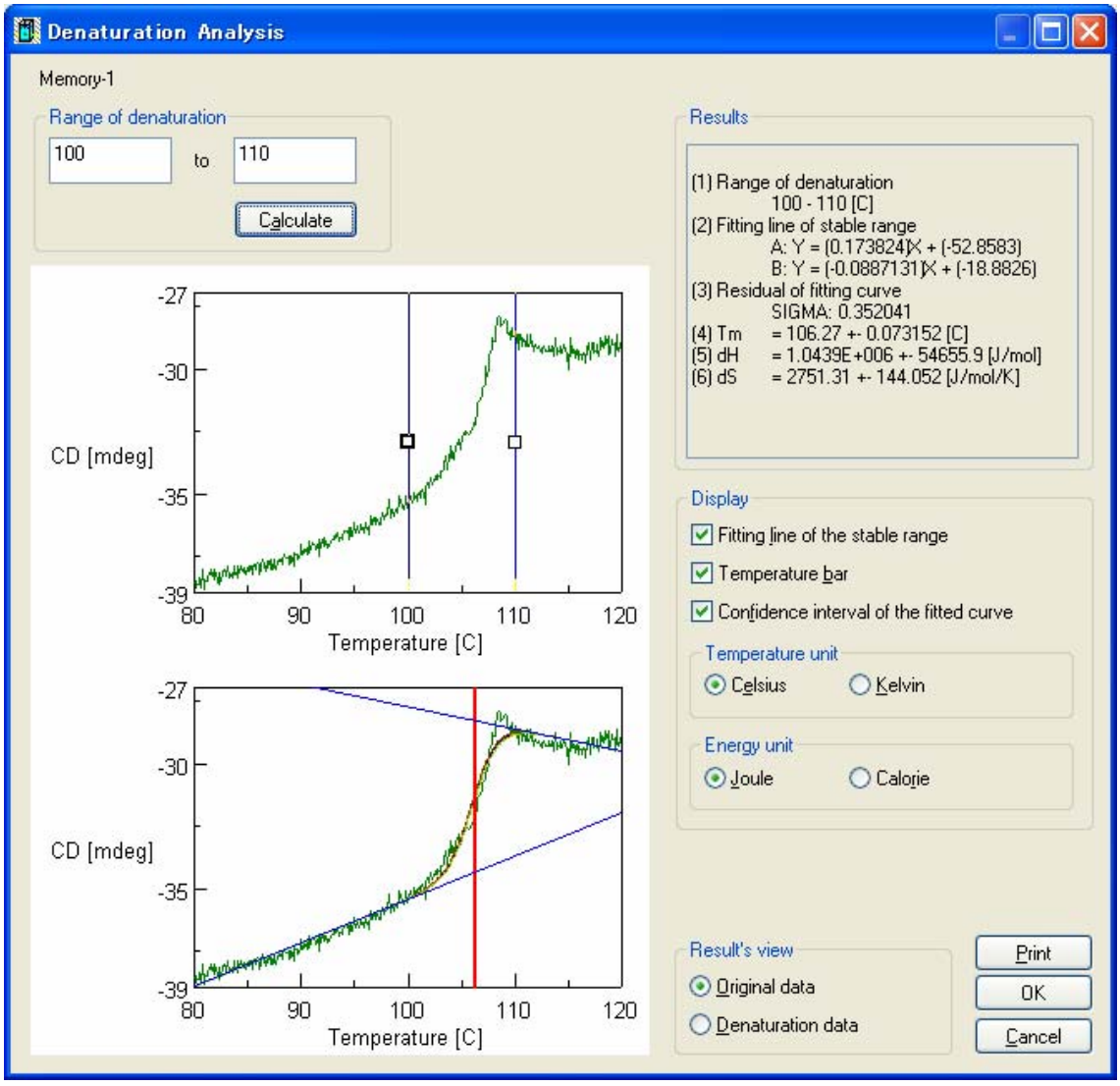

Fig. 2 Thermal denaturation analysis results of super thermostable cellulase

<Reference>

(1) S. Uchiyama, A. Ohshima, S. Nakamura, J. Hasegawa, N. Terui, S. J. Takayama, Y. Yamamoto, Y. Sambongi, and Y. Kobayashi, *J. Am. Chem. Soc.* **2004**, *126*, 14684-14685.

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