

T_m analysis of multiple samples using High-Throughput Circular Dichroism (HTCD) system

Introduction

In CD measurements of proteins, thermodynamic parameters obtained by thermal denaturation analysis provide important information about proteins along with results of secondary structure estimation.

JASCO's J-800 HTCD system enables researchers to automate not only CD spectra measurements and secondary structure estimation but also variable temperature measurements and thermal denaturation analysis of multi samples.

We introduce variable temperature measurement data and results from three proteins measured using the HTCD system and the [Thermal Denaturation Multi Analysis] program.

Keywords: T_m analysis, Multiple samples, HTCD

<System>

J-815 CD Spectrometer
ASU-800 Auto sampler

ASP-849 Syringe pump
JFLC-498 Peltier type flow cell

<Samples>

Proteins: Human Serum Albumin (HSA), Lysozyme (Lyz), Ribonuclease (RNaseA)
Concentration: 0.2 mg/mL H₂O

<Parameters>

Pathlength:	2 mm	Temp.:	20-85°C	Rate:	1 °C/min
Wavelength:	222 nm	Bandwidth:	0.2 nm	Response:	4 sec
Data interval:	0.2°C				

<CD stability>

The flow cell of the HTCD system is designed to prevent the influence of temperature increases on baseline measurements. As shown in Fig. 1, CD value of a solvent blank (H₂O) is stable in the 20 - 90°C region. HTCD system realizes highly precise measurements.

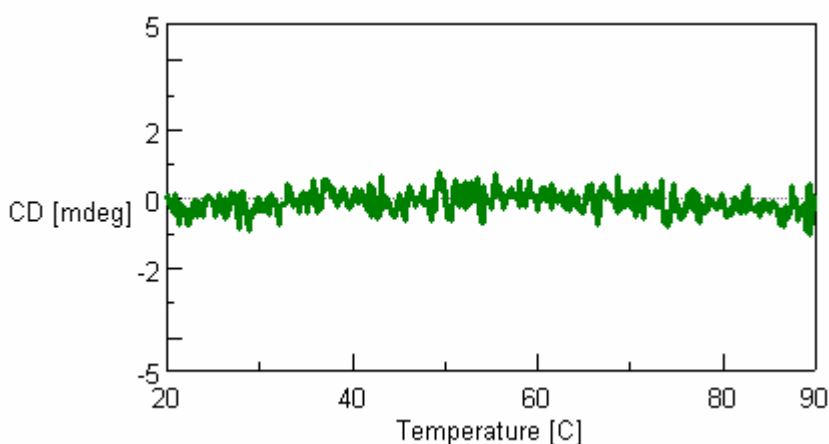


Fig. 1 CD stability of HTCD system when measuring H₂O blank

<Thermal Denaturation Multi Analysis>

The analysis results of the variable temperature measurement data of HSA, Lyz, and RNaseA are shown below. Thermodynamic parameters can be calculated using the [Thermal Denaturation Multi Analysis] program for all data in a selected folder.

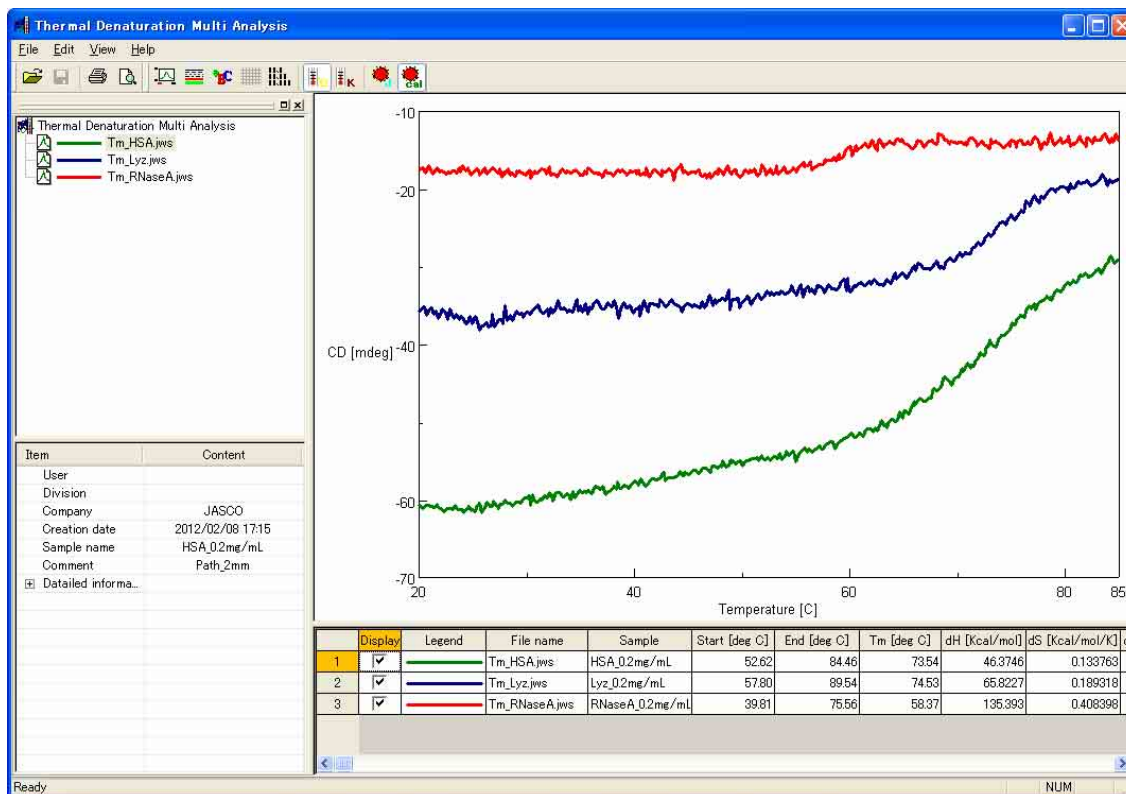


Fig. 2 [Thermal Denaturation Multi Analysis] program

Table 1 Thermodynamic parameters of three proteins measured using the HTCD system

Sample name	Tm (°C)	dH (kcal/mol)	dS (kcal/mol/K)
Human Serum Albumin (HSA)	73.5	46.4	0.134
Lysozyme (Lyz)	74.5	65.8	0.189
Ribonuclease (RNaseA)	58.4	135.4	0.408