

CD measurement of microscale sample by J-1500 CD spectrometer - Measurement of hemoglobin and DNA derived from bovine thymus -

Introduction

CD measurement method is an essential tool for structural analysis of biological molecule such as protein or nucleic acid. In case of using the cell with the optical pathlength of 1mm in CD measurement, it is possible to measure the microscale sample as low as 400 μl in volume and 0.1 mg/ml in concentration, however for the precious samples, there may be the case that it is difficult to get even such amount of samples.

J-1500 CD spectrometer enables the measurement of CD spectra with high accuracy and high S/N ratio of even microscale sample using the micro cell with small acceptance surface, due to the improved optical system for higher throughput and the electrical system using the latest digital lock-in detection.

In this application data, CD measurement of hemoglobin and DNA derived from bovine thymus was implemented using micro sampling disk for microscale sample and J-1500 CD spectrometer. The measurement was performed with sample volume of 3 μl , and secondary structural analysis was performed for CD spectra of hemoglobin.

Keywords: Microassay, Micro sampling disk, Biological molecule

Measurement condition

Measurement unit:	J-1500 CD Spectrometer		
	Micro sampling disk, Cylindrical cell		
Data sampling interval:	0.1 nm	Response:	2 seconds
Spectral bandwidth:	1 nm	Scan speed:	100 nm/min
Optical pathlength:	0.2 mm	Concentration:	0.5 mg/ml
Accumulation:	9 times (Micro sampling disk)		
	1 time (Cylindrical cell)		



* By simply dropping the sample in the central part of the disk, the sample is surrounded by water repellent, and so the sample can be retained in optical axis even when the disk assembly is placed at vertical position in sample compartment.

Fig. 1 Procedure of micro sampling disk

Measurement results

CD spectra of hemoglobin and DNA derived from bovine thymus by using of micro sampling disk and normal cylindrical cell are shown in Fig. 2 and Fig. 3. Accurate CD spectra with high S/N ratio were obtained in the range down to 180 nm even with sample volume of only 3 μ l.

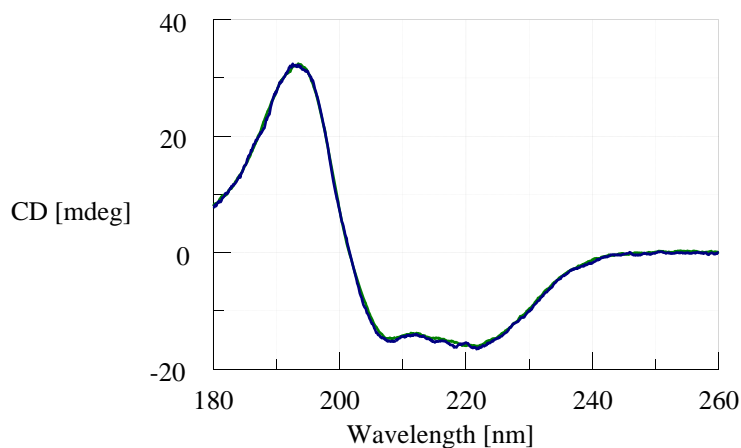


Fig. 2 CD spectra of hemoglobin

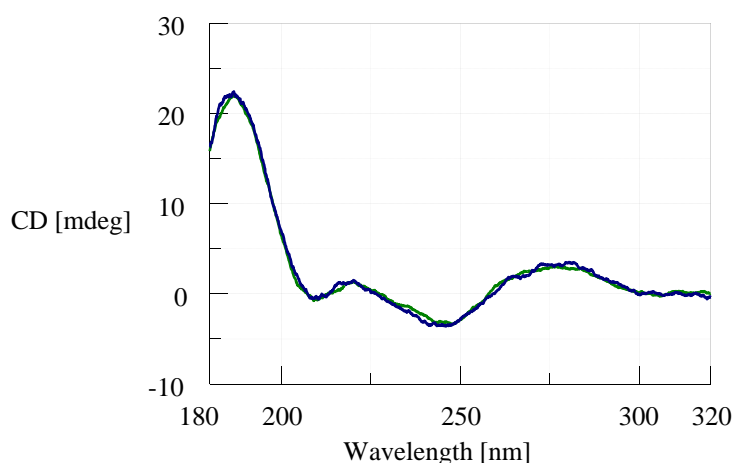


Fig. 3 CD spectra of DNA derived from bovine thymus

— : Micro sampling disk
— : Cylindrical cell

Secondary structural analysis

For CD spectra of hemoglobin measured by using the micro sampling disk and cylindrical cell, the secondary structural analysis by JWMVS-529 CD multivariate SSE analysis program was performed. The analysis results comparing with the result by X-ray crystal structural analysis are shown in Table 1. The secondary structural analysis result by CD spectra obtained using micro sampling disk was in good agreement with the result by X-ray crystal structural analysis as well as the result by CD spectra obtained using normal cylindrical cell.

Table 1 Comparison of secondary structural analysis

		Helix (%)	β -Sheet (%)	Turn (%)	Random (%)
Hemoglobin	X-ray	78.0	0.0	6.3	15.7
	Micro- sampling disk	73.8	0.0	10.9	15.3
	Cylindrical- cell	72.2	0.0	12.0	

1) The results of X-ray crystal structural analysis refer to DSSP data included to RCSB PROTEIN DATA Bank (PDB).

Hemoglobin: 2qss
Helix: (α -helix) + (3/10-helix)
 β -sheet: β -strand
Turn: turn
Random: other

2) JWMVS-529 CD multivariate SSE analysis program used here for analysis was by using PCR method.