Application Note

CD-0017

CD spectral measurement of protein water solution in vacuum UV region by J-1500 CD spectrometer

Introduction

CD spectroscopy is an essential tool for structural analysis of proteins. CD spectra reflecting the secondary structure of protein are usually observed from UV region below 260 nm to the vacuum ultraviolet region, however, the water itself used as a solvent has strong absorption in the vacuum UV region. In case of light water (H_2O), its absorption becomes drastically higher at around 175 nm and even the absorption of heavy water (D_2O), at around 170 nm. Therefore, in order to obtain the highly accurate SSE results, the measurement keeping high signal to noise ratio (S/N) down to such wavelength limit for water is required.

The JASCO's new J-1500 CD spectrometer allows the researchers to carry out the measurement with high S/N ratio in the vacuum UV region, by incorporating the several latest technologies such as digital lock-in detection in electrical system, high-throughput optics and highly effective nitrogen gas purging system based on computational fluid simulation.

In this application note, the CD measurement of protein water solution (light water: H_2O) down to the vacuum UV region was performed in order to carry out the SSE, by using of the J-1500 CD spectrometer and multivariate SSE program (Model JWMVS-529).

Keywords: Secondary structure estimation (SSE), CD multivariate SSE, improvement of estimation accuracy of β-sheet structure

Measurement Conditions

Apparatus:	J-1500 CD spectrometer	Data acquisition interval:	0.1 nm
Response:	2 sec	Spectral bandwidth (SBW):	1 nm
Scan speed:	50 nm/min	Accumulation:	4
Sample concentration:	1 mg/mL (H_2O)	Cell pathlength:	0.1 mm (cylindrical quartz cell)

Results

CD spectra of human serum albumin (Helix rich), concanavalin A (β-sheet rich) and trypsin inhibitor (Random rich) in light water solution (H_2O) are shown in Fig. 1. CD spectrum reflecting each specific secondary structure was obtained for each protein down to 174 nm with high S/N ratio.

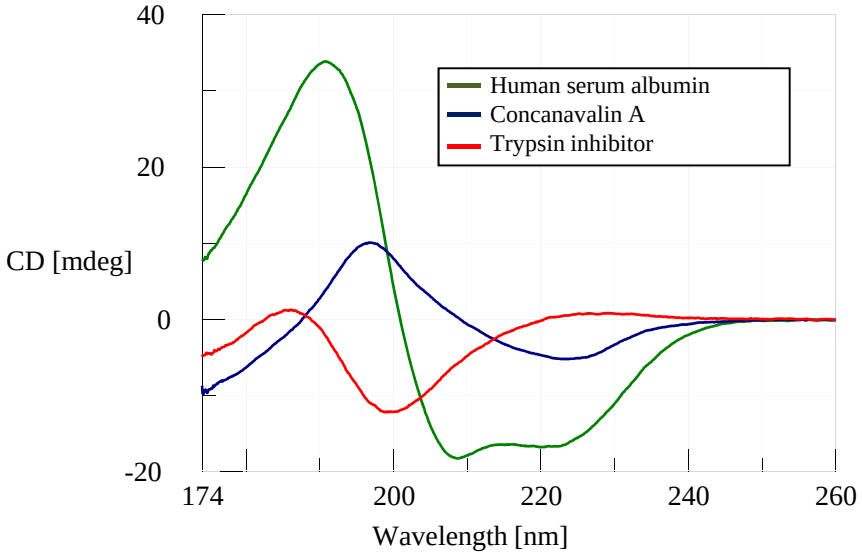

Fig. 1. CD spectra of protein water solution in vacuum UV region

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Secondary structure estimation (SSE)

SSE was carried out for spectrum of each protein by JASCO's multivariate SSE program (JWMVS-529) and the results are shown in Table 1 together with each corresponding SSE result by X-ray crystal structural analysis and CDSSTR method for comparison. Regarding the SSE by CD spectrum, it is generally known that the estimation of β-sheet structure is less accurate than that of Helix structure, however in Table 1, as you can clearly see, the better estimation of b-sheet structure was obtained by using JASCO's multivariate SSE program, referring to the results by X-ray. This improvement was achieved by applying PLS/PCR multivariate analysis method and calibration model generated by CD spectra of 26 kinds of standard protein in the range from 260 to 176 nm.

Table 1 SSE results by three different methods

		Helix (%)	β-Sheet (%)	Turn (%)	Random (%)
Human serum albumin	X-ray [1)]	71.8	0.0	8.2	20.0
	JASCO [2)]	70.6	0.0	9.4	20.0
	CDSSTR [3)]	71.1	0.0	6.9	22.9
Concanavalin A	X-ray	3.8	46.4	10.5	39.2
	JASCO	9.7	42.7	10.4	37.2
	CDSSTR	6.1	35.3	12.0	46.6
Trypsin inhibitor	X-ray	1.7	33.1	10.5	54.7
	JASCO	1.0	35.8	14.6	48.6
	CDSSTR	5.1	17.5	16.2	59.5

1) The SSE results by X-ray crystal structural analysis are all referred to the data of RCSB PROTEIN DATA Bank (PDB).

(protein)	(PDB ID)
Human serum albumin:	1bm0
Concanavalin A:	2ctv
Trypsin inhibitor:	1ba7

Helix: (α-helix) + (3/10-helix)
β-Sheet: β-strand
Turn: turn
Random: other

2) In JASCO's multivariate SSE program (JWMVS-529), PLS/PCR method is applied to:

Human serum albumin:	PLS
Concanavalin A:	PLS
Trypsin inhibitor:	PCR

3) In CDSSTR method, the reference spectra "SP22X[H, 3/10, S, T, P2, U]178-260 nm" are used:

Helix: (α-helix) + (3/10-helix)
β-Sheet: β-sheet
Turn: turn
Random: (poly(Pro)II Structure) + (unordered)

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