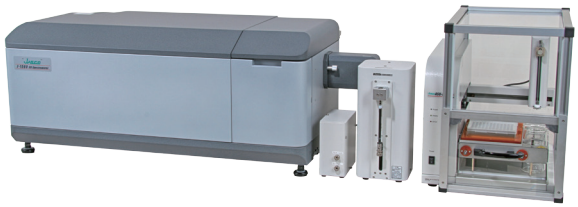
Application Note
CD Spectroscopy

Using the Multivariate Secondary Structure Estimation (SSE) program

Introduction

CD measurements reveal information regarding the secondary structure or backbone conformation of a protein. Determination of the secondary structure content of a biomolecule can elucidate the relationship between structure and function or verify protein stability. JASCO's multivariate Secondary Structure Estimation (SSE) program can be used to analyse CD data in order to supply researchers with more quantitative structure estimations. A large number of samples can be automatically measured and efficiently analysed using the High-Throughput CD (HTCD) system and multivariate SSE program.

This application notes demonstrates secondary structure results of eight proteins using the Multivariate SSE program.



JASCO J-1500 high-throughput CD system
View product information at www.jascoinc.com

Keywords

Secondary structure estimation, circular dichroism, HTCD, Multivariate, Biochemistry, Pharmaceuticals

Experimental

Measurement conditions			
Data acquisition interval	0.1 nm	Response time	2 seconds
Spectral bandwidth	1 nm	Scan speed	100 nm/min
Accumulations	2 times	Path length	1 mm

Results

Prior to obtaining the sequence measurement, the pathlength and mean residue molar concentrations have to be specified so that the optical constants can be automatically calculated for the secondary structure estimation (Figure 1).

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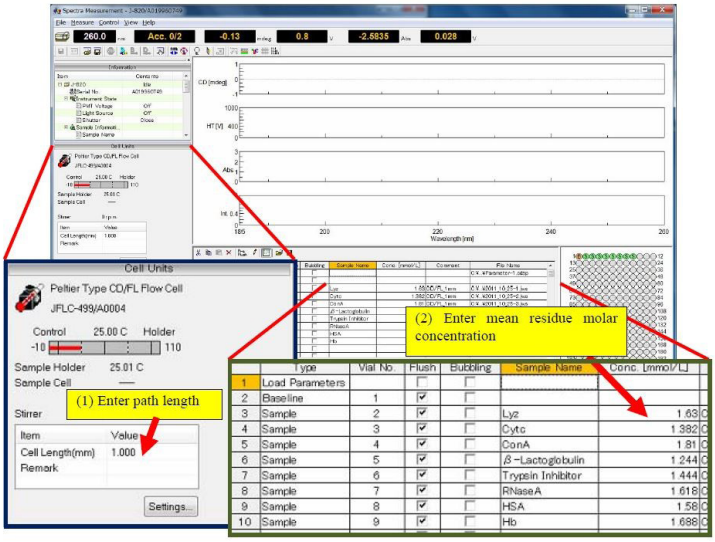

Figure 1. Multivariate parameter setup specifying path length and mean residue molar concentration

The CD measurements in the far-UV region are obtained for eight 0.1 mg/mL protein samples with varying secondary structure compositions and are shown in Figure 2.

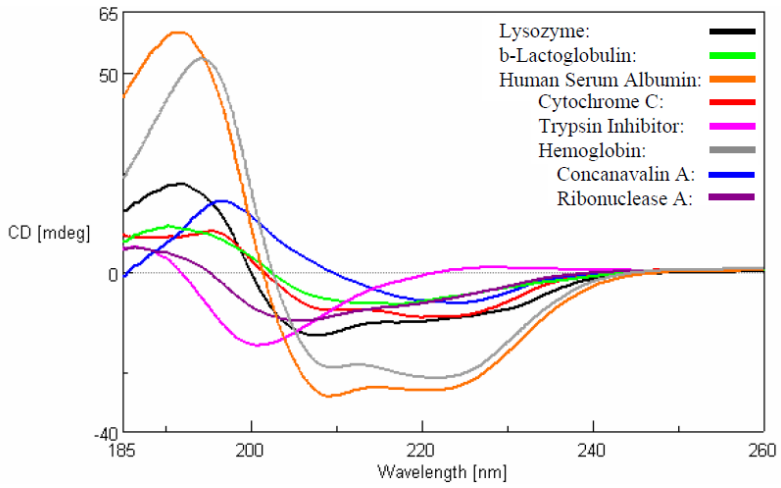

Figure 2. CD spectra of protein samples

The secondary structure is estimated from the protein spectra in Figure 1 using the PLS multivariate method. Table 1 compares these results with X-ray crystallography data. Figure 3 shows the secondary structure results set up in the JWMVS program.

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Table 1. Comparison of Multivariate SSE results with X-ray crystallography data

		α-Helix %	β-Sheet %	Turn %	Other %
Lysozyme	PLS	42.8	0.4	24.4	32.4
	X-ray[1]	41	4	19	35
Cytochrome C	PLS	42.6	3.1	18.1	36.2
	X-ray[1]	42	8	9	42
Concanavalin A	PLS	5.1	44.6	13.9	36.4
	X-ray[1]	2	36	12	49
β-Lactoglobulin	PLS	17.8	35.5	12.3	34.4
	X-ray[1]	13	34	13	41
Trypsin inhibitor	PLS	13.9	25.3	17.3	43.5
	X-ray[2]	2	33	10	55
Ribonuclease A	PLS	21.5	14.7	22.4	41.4
	X-ray[1]	22	19	11	48
Human Serum Albumin (HSA)	PLS	66.8	1.3	8.2	23.7
	X-ray[2]	72	0	8	29
Hemoglobin	PLS	61.1	0	18	20.9
	X-ray[1]	75	0	10	15

[1] W. C. Johnson, *Proteins: Structure, Function, and Genetics* (1999), 35, 307-312.
[2] PDB: Trypsin inhibitor: 1ba7 (DSSP), HAS: 1bm0 (DSSP)

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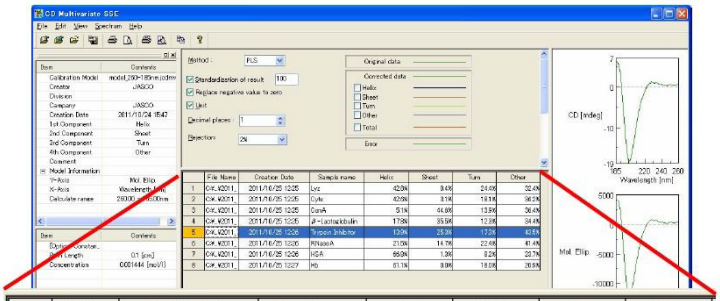

	File Name	Creation Date	Sample name	Helix	Sheet	Turn	Other
1	C:¥...¥2011_	2011/10/25 12:25	Lyz	42.8%	0.4%	24.4%	32.4%
2	C:¥...¥2011_	2011/10/25 12:25	Cytc	42.6%	3.1%	18.1%	36.2%
3	C:¥...¥2011_	2011/10/25 12:25	ConA	5.1%	44.6%	13.9%	36.4%
4	C:¥...¥2011_	2011/10/25 12:25	β-Lactoglobulin	17.8%	35.5%	12.3%	34.4%
5	C:¥...¥2011_	2011/10/25 12:26	Trypsin Inhibitor	13.9%	25.3%	17.3%	43.5%
6	C:¥...¥2011_	2011/10/25 12:26	RNaseA	21.5%	14.7%	22.4%	41.4%
7	C:¥...¥2011_	2011/10/25 12:26	HSA	66.8%	1.3%	8.2%	23.7%
8	C:¥...¥2011_	2011/10/25 12:27	Hb	61.1%	0.0%	18.0%	20.9%

Figure 3. Results set up using the Multivariate SSE program

Conclusion

This application note illustrates the use of the Multivariate SSE program in obtained secondary structure data after CD measurements. The PLS method shows good agreement with secondary structure results obtained by X-ray crystallography.

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