Application Note

No. 210TR0177-E

Secondary Structure Analysis of Proteins using FTIR

Introduction

Recently, it was reported that the identification of human DNA base sequence had been completed. Since base sequence was identified, it has been said that the studies of protein are going to increase. In the protein research, the studies of the function and structure are needed to be done concurrently. Therefore JASCO has developed new program, which can analyze the secondary structure of protein utilizing the peak of amido I in IR spectrum (hereinafter referred to as IR-SSE). In the conventional analysis of the secondary structure of protein using IR spectrum, deuterated water (D_2O) was used for prevention against overlapping of the peaks of water (H_2O) to amido I. However, there was a problem that deuterated water is much more expensive than common water and also difficult to use. IR-SSE has been so designed that the peak of water is subtracted automatically in order to analyze the secondary structure of protein using common water. The secondary structure had been analyzed from amido I peak using the curve fitting method, however, the current program enables the secondary structure analyses of protein by the method of principal-component regression (PCR) or partial least squares (PLS), which take 17 kinds of proteins whose secondary structure was known as standard data. The results of the analyses of 5 proteins using IR-SSE are shown.

Experimental

Sample:	5 kinds of 5% (w/v) proteins (Lysozyme, Ig-G, Cyt-C, OVA, Pepsine), in Phosphate buffer (10mM pH 7.0) were prepared.
Measurement method:	Transmittance measurement (10 mL samples were sandwiched between 2 CaF_2 plate.)
Instrument:	FTIR-680
Measurement condition:	Accumulations: 32 times Resolution: 4 cm^{-1} Apodization: Cosine The measurement was done 3 times on above conditions.

Results

Table 1 shows the results of secondary structures of 5 proteins. The repetitive reproducibility was considered to be quite good, however, there was little difference from the data using X-ray. It can be explained as this distinction was occurred from the difference of conditions between crystal and solution, and that of measured proteins (including purity and bioracial tissues). Therefore, this program is sufficiently applicable to the analyses of the secondary structure of proteins.

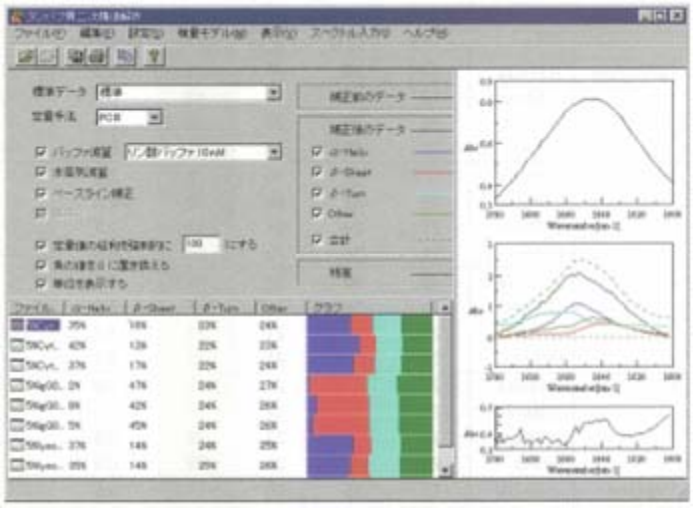

Fig. 1 Screen of IR-SSE

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Table 1 The comparison of reproducibility with the analysis of FT/IR and X-ray (values of reference)

		First	Second	Third	Avarage	Relative Standard Deviation	X-rays
Lysozyme	αHelix	37	38	40	38	1.3	36.0
	βSheet	18	18	17	18	0.2	10.0
	βTurn	28	28	27	28	0.9	36.0
	Other	28	28	27	27	0.5	19.0
Ig-G	αHelix	7	12	10	10	2.5	3.0
	βSheet	45	42	45	44	2	67.0
	βTurn	24	24	21	23	1.7	18.0
	Other	24	25	23	24	0.9	12.0
Cyt-C	αHelix	38	40	42	40	2.1	42.0
	βSheet	19	16	15	16	2.5	2.0
	βTurn	24	23	21	23	1.2	22.0
	Other	23	22	21	22	1.1	34.0
OVA	αHelix	27	28	24	26	1.9	N.D.
	βSheet	32	31	32	32	0.5	N.D.
	βTurn	28	28	27	28	0.6	N.D.
	Other	29	29	28	29	0.8	N.D.
Pepsine	αHelix	15	15	16	15	0.5	N.D.
	βSheet	28	28	27	28	0.6	N.D.
	βTurn	23	22	21	22	1.5	N.D.
	Other	24	22	21	22	1.6	N.D.

The unit is %. N.D. means no data.

Reference

1) Ronald W. Sarver et al., Analytical Biochemistry 194, 89-100 (1991)