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Application News

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IRTracer™-100 / FTIR Analysis / Protein Structure

Determination of Protein Secondary Structures using FTIR Spectroscopy

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❑Introduction

Proteins can perform a wide variety of molecular functions due to their unique conformation. There are two different representations encoding the structural conformation of proteins, which are the secondary structure and tertiary structure. The secondary structure of proteins is characterized by the repetitive folding pattern of helices, sheets, turns and disordered coils [1]. Different techniques such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy have been widely used to investigate the conformation and stability of secondary structure in proteins. However, these techniques are costly and require specific and time-consuming sample preparation steps.

Fourier transform infrared (FTIR) spectroscopy is suitable to determine the secondary structure of proteins due to its versatility and high sensitivity towards chemical composition of molecules. The secondary structure composition of proteins could be obtained from the amide band I ranging from 1600 cm^{-1} to 1700 cm^{-1} of the IR spectrum. Each type of the secondary structure has its specific spectral band which overlaps in this region [2]. Mathematical procedures such as band curve-fitting and second derivatives can be applied to resolve the overlapping amide I band components and to quantify the secondary structure of proteins. In this application news, we examine the secondary structure of proteins by using FTIR transmission spectroscopy and band curve-fitting data analysis.

❑ Experimental

Three proteins, lysozyme, myoglobin and concanavalin A, were purchased from Sigma Aldrich, USA. The aqueous solutions of the protein were prepared in 20 mM sodium phosphate buffer, pH 7.2. The concentration of protein solution prepared was 15 mg/mL. For FTIR transmission measurement, a Specac Omni-Cell liquid cell with calcium fluoride (CaF_2) windows and a 6 µm path length Mylar spacer were used.

The reference blank which is the sodium phosphate buffer was introduced through the filling port of the cell where the solution is sandwiched between the CaF_2 windows. Infrared spectrum was obtained using Shimadzu IRTracer™-100 FTIR spectrometer and the measurement conditions are shown in Table 1.

Table 1: Instruments and analytical conditions

Instruments	: IRTracer™-100
Resolution	: 4.0 cm^{-1}
Accumulation	: 100
Apodization	: Happ-Genzel
Detector	: DLATGS

The IR spectra were acquired in the wavenumber range of 4000 cm^{-1} to 1000 cm^{-1}. The same procedure was conducted for the protein solution. Due to the short pathlength of the cell, only a small volume of protein solution is required for each measurement. Each sample was measured four times.

❑Results and Discussion

Water has a strong absorption in the protein amide I region at around 1645 cm^{-1}. The reference spectrum was subtracted from the protein solution spectrum to remove the bands from water so as to obtain the true protein spectra. To prevent over- or under-subtraction of the buffer spectrum, a flat baseline should be obtained from 2000 cm^{-1} to 1700 cm^{-1} without a negative lobe in the range. Figure 1 shows the overlay IR spectra of protein samples after subtraction.

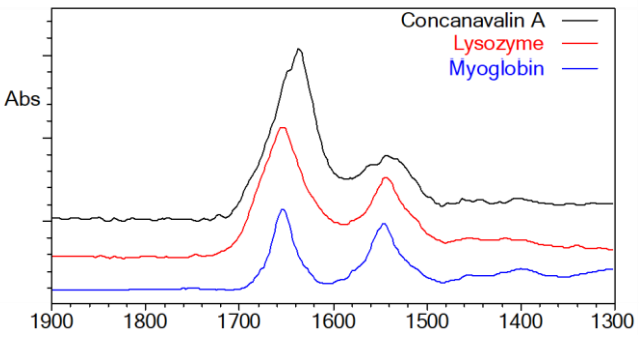

Figure 1: Overlay IR spectra of concanavalin A, lysozyme and myoglobin after subtraction of buffer solution

The resulting protein difference spectra were smoothened using a seven-point Savizky-Golay function to remove any possible white noise. Amide band I (1600 – 1700 cm^{-1}) was baseline-corrected. LabSolutions™ IR Curve-Fitting Program was used for band curve-fitting analysis. Band curve-fitting analysis of the amide band I was performed using Gaussian function. The band assignments in the amide band I region for each protein was based on Table 2 [3]. Figure 2 shows the IR spectra of curve-fitted amide band I for concanavalin A (A), lysozyme (B) and myoglobin (C).

The band area for each component peak was used to calculate the relative contribution of component to a particular protein secondary structure. Table 3 summarizes the quantitation results of α-helix, β-sheet, β-turns and random secondary structures for all three proteins. The quantitation results are averaged from four repeated measurements. The calculated results for all three proteins are fairly comparable with the X-ray crystallography results [4].

Table 2: Secondary structure band wavenumber and assignments for protein in water

Secondary Structure	Band Assignment in Water (cm^{-1})
α-helix	1654.0 – 1658.0
β-sheet	1624.0 – 1642.0 1691.0 – 1696.0
β-turn	1666.0 – 1688.0
Random	1646.0 – 1650.0

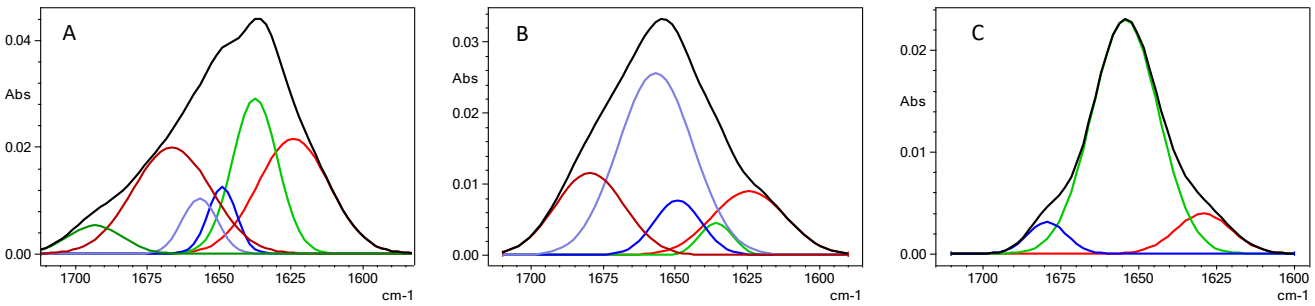

Figure 2: IR spectrum of concanavalin A (A), lysozyme (B) and myoglobin (C) with the fitted component bands

Table 3: Comparison of protein secondary structure calculation determined by FTIR and X-ray crystallography

Secondary Structure (%)	Concanavalin A		Lysozyme		Myoglobin	
	FTIR	X-Ray	FTIR	X-Ray	FTIR	X-Ray
α-helix	6.0	3.0	49.1	45.0	80.0	85.0
β-sheet	61.0	60.0	16.0	19.0	13.6	0.0
β-turn	25.6	22.0	19.6	23.0	6.4	8.0
Random	7.5	15.0	15.3	13.0	0.0	7.0

❑ Conclusions

Transmission measurement using FTIR spectroscopy and band curve-fitting analysis is a feasible method to quantify secondary structure of proteins in aqueous solution with minimal sample volume and simple sample preparation step. The quantitation results for different secondary structure determined from band curve fitting of amide band I region are comparable to the results obtained through X-ray crystallography.

❑ References

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