SHIMADZU APPLICATION NEWS

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● SPECTROPHOTOMETRIC ANALYSIS

No.A388

Analysis of Protein Secondary Structure Using FTIR

Techniques such as X-ray diffraction and NMR are often used for the structural analysis of proteins. Infrared spectrometry is also used for the analysis of items related to the secondary structure, such as the α helix and β sheet. Infrared spectrometry allows simple measurement regardless of the state of the sample (e.g., solid or liquid, crystalline or amorphous) and so it is used as a supplement to the above techniques. Information about the secondary structure of proteins that have infrared spectra is presented here.

■ Infrared Spectra of Proteins

A transmission spectrum of bovine serum albumin is shown in Fig.1. The peak near 1,650 cm^{-1} corresponds to the C=O stretching vibrations in peptide bonding and is called the "amide Ⅰ band". Similarly, the peaks near 1,540 cm^{-1} (N-H bending vibrations and C-N stretching vibrations) and 1,240 cm^{-1} (C-N stretching vibrations and N-H bending vibrations) are the amide Ⅱ and amide Ⅲ bands, respectively. Also, the peak near 3,300 cm^{-1} probably corresponds to N-H stretching vibrations and the peak near 1,400 cm^{-1} probably corresponds to the protein side-chain COO^-. Among these representations of protein-based absorbance, the peak position and shape of the amide Ⅰ band varies with the secondary structure and so information about the secondary structure can be obtained by analyzing this peak.

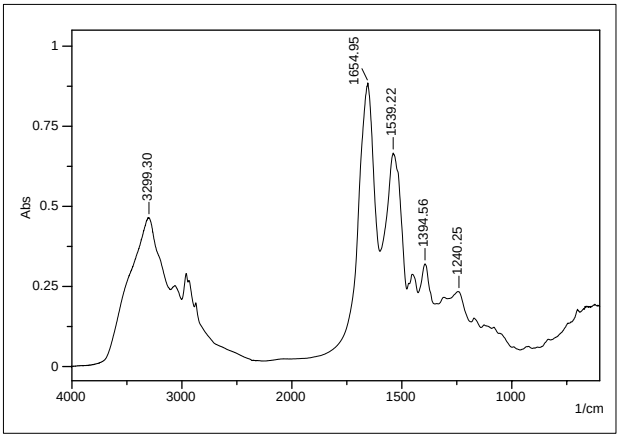

Fig.1 Spectrum of Bovine Serum Albumin

■ Spectra of Lysozyme and Ribonuclease A

Examples of representative protein secondary structures include α helixes, which have helical structures, β sheets, which have linear structures, and loops, which have irregular lengths and structures. The amide Ⅰ absorbance is different for each. In aqueous solutions, α helixes have absorption near 1,650 cm^{-1}, β sheets have absorption near 1,630 cm^{-1}, and loops have absorption near 1,645 cm^{-1} (broad).

Fig.2 shows the amide Ⅰ band of Lysozyme and Ribonuclease A in deuterium water. (The spectra were obtained by subtracting the portion corresponding to deuterium water.) Refer to Table 1 for the measurement conditions. The peak tops of Lysozyme and Ribonuclease A are at 1,651 cm^{-1} and 1,640 cm^{-1} respectively. Both substances, however, are composed of multiple secondary structures and so these peak formations actually represent the aggregates of multiple peaks.

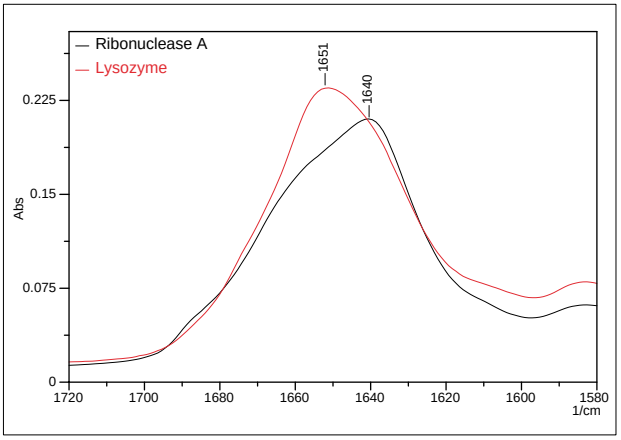

Fig.2 Spectra of Lysozyme and Ribonuclease A

Fig.3 shows the result of applying secondary differentiation to the spectra shown in Fig.2. This makes it possible to clarify the positions of overlapping peak. It can be seen here that Lysozyme has a large number of α helixes and Ribonuclease A has a large number of β sheets.

Table 1 Analytical Conditions

Method	: Between BaF_2 (0.01 mm)
Resolution	: 2 cm^{-1}
Accumulation	: 100 (approx. 4 minute)
Detector	: DLATGS

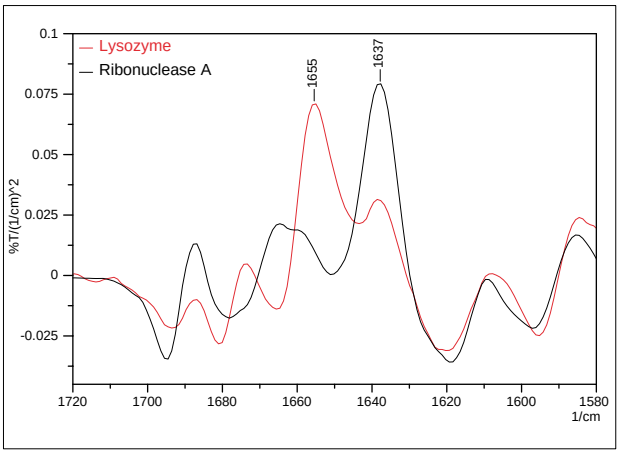

Fig.3 Secondary Differential Spectra of Lysozyme and Ribonuclease A

■ ATR spectra of Albumin

Fig.4 shows the amide Ⅰ band measured for two types of Albumin (Egg albumin and Human serum albumin) using single-reflection ATR (with a diamond prism). Fig.5 shows the result of applying secondary differentiation to the spectra shown in Fig.4. Refer to Table 2 for the measurement conditions.

It can be inferred from these results that, for Egg albumin, absorption is strong near 1,633 cm^{-1} and there are large number of β sheets and, for Human serum albumin, absorption is strong near 1,651 cm^{-1} and there are large number of α helixes.

This analysis was performed with the help of Prof. Masanosuke Nara of the College of Liberal Arts and Sciences, Tokyo Medical and Dental University.

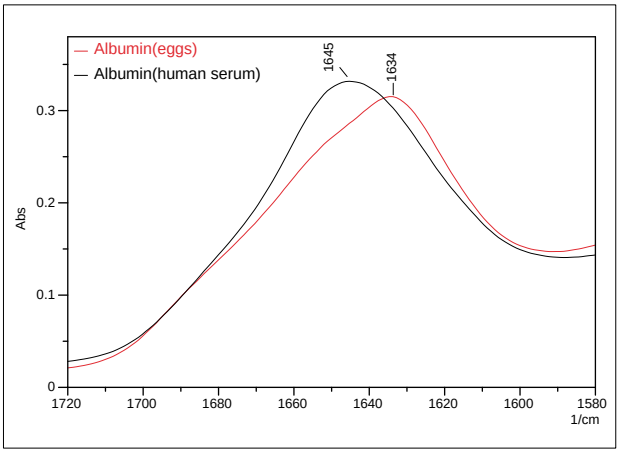

Fig.4 ATR Spectra of Albumin
(Red: Egg Albumin; Black: Human Serum Albumin)

Table 2 Analytical Conditions

Attachment	: DuraSamplⅡR
Resolution	: 2 cm^{-1}
Accumulation	: 100 (approx. 4 minute)
Detector	: DLATGS

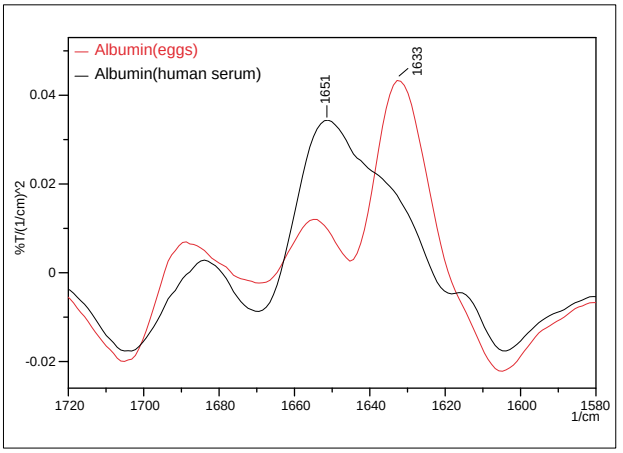

Fig.5 Secondary Differential Spectra of Albumin
(Red: Egg Albumin; Black: Human Serum Albumin)

SHIMADZU

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