Application Note

210-TR-0127

Measurement of Protein in Heavy Water by FT-IR

<Introduction>

Within the past decade, analyzing protein sequences consisting of 30 peptides or fewer has become very common. The number of peptide hormones that have been produced by peptide synthesis has become very large. As a result, the need to evaluate these hormones using analytical instruments has increased rapidly. This application bulletin demonstrates FT/IR measurement of several types of protein in heavy water. It is well-known that in the IR spectrum of a protein, the characteristic vibration peaks of the principal chain appear in the range of 1700 - 1600 cm^{-1} (approximately 6 µm) for amide I, and in the 1600 - 1500 cm^{-1} range (approximately 6.45 µm) for amide Ⅱ. If IR measurement of protein is conducted in an aqueous solution, the strong absorption band of normal water occurring at 6 µm prevents meaningful date acquisition. In order to overcome this problem, it is necessary to measure the protein in heavy water. When the protein is immersed in heavy water, the sample can be measured in affixed cell of 50 - 100 µm in width. In this case, a waterproof cell window must be sued (Table 1). In transmittance mode, CaF_2 or BaF_2 are typically used; ZnSe, which is typically employed for ATR, can be used as well. These window materials are transparent, offering the advantage of easy detection of air bubbles.

<Condition>

Resolution: 2 cm^{-1}
Detector: TGS
Apodization: Cosine
Accumulation: 256

Sample preparation
Solution: Heavy water
Protein concentration: 2% (w/v)
Cell window: CaF_2
Cell thickness: 0.1 mm (fixed cell)

Table 1

Material	Chemical formula	Limited to Low wavenumber (cm-1)	Note
Potassiume fluoride	CaF_2	1100	For trancemittance
Bariume fluoride	BaF_2	750	For trancemittance
Zinc selenide	ZnSe	625	Trancemittance / ATR
Arsenic selenide	As_2Se_3	650	Specify of poison
Germanium	Ge	830	For ATR

Note: When the amount of available sample is small, or when the sample is expensive, we recommend that the demountable cell be used. If the fixed cell is used for such samples, air bubbles trapped in the cell can make the results meaningless, thus wasting the sample. In addition, the instrument should be allowed sufficient time to stabilize after the power is turned on, and the interval between the measuring sample and the blank should be as short as possible.

<Measurement data>

We measured five protein samples: whale Myoglobin, Lysozyme from the while of the chicken egg, Ribonuclease A from the bovine liver, Cytochrome C from the horse heart, Bovine serum albumin(SIGMA). Sample measurement was performed using a CaF_2 cell measuring 0.1 mm in thickness after 8 mg of each protein was dissolved in 0.4 mL of heavy water and allowed to sit for 24 - 48 hours for deuterium substitution. The results are shown in Figure 1 - 6. Figure 2 - 6 show the spectra of each protein after subtraction of the deuterium spectrum, and then smoothing. Figure 1 shows the overlaid spectra of 2 % myoglobin in heavy water, and heavy water alone. Using a cell measuring 0.1 mm in thickness, the usable wavenumber range of the heavy water solvent is 2100 - 1300 cm^{-1} because the absorbance of the solvent is lower than 1. Deuterium substitution causes the band of Amide II, which normally appears around 1550 cm^{-1}, to shift to a much lower wavenumber. Therefore, only the Amide I appearing at 1650 cm^{-1} absorption band of the principal chain observed.

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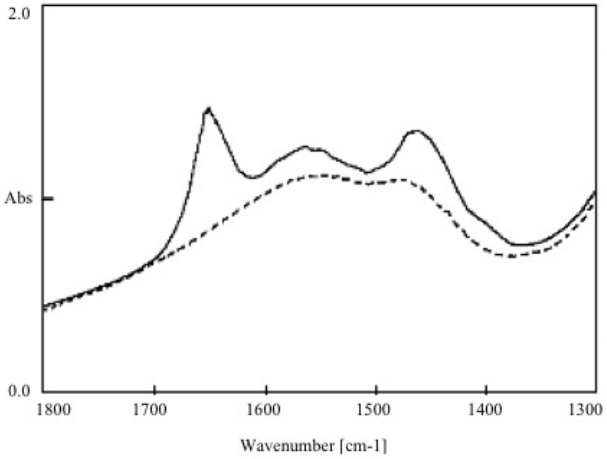

Figure 1 Overlaid spectra of 2% myoglobin in Heavy water, and Heavy water

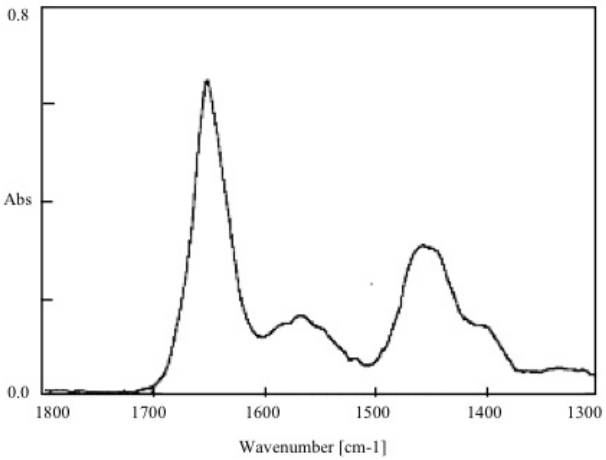

Figure 2 Myoglobin

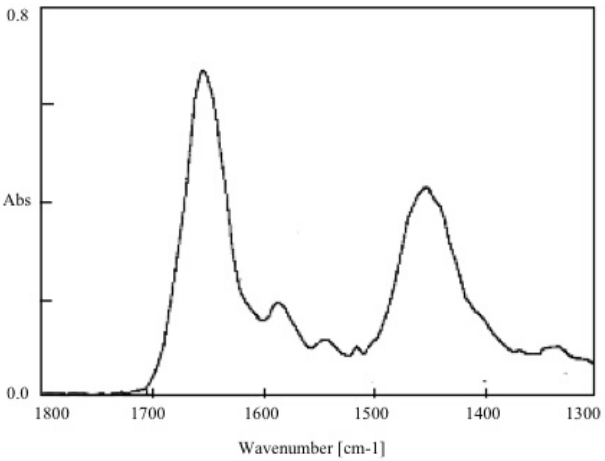

Figure 3 Lysozyme

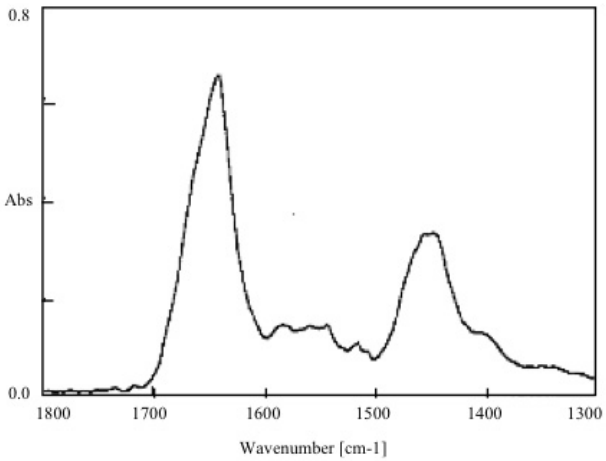

Figure 4 Ribonuclease

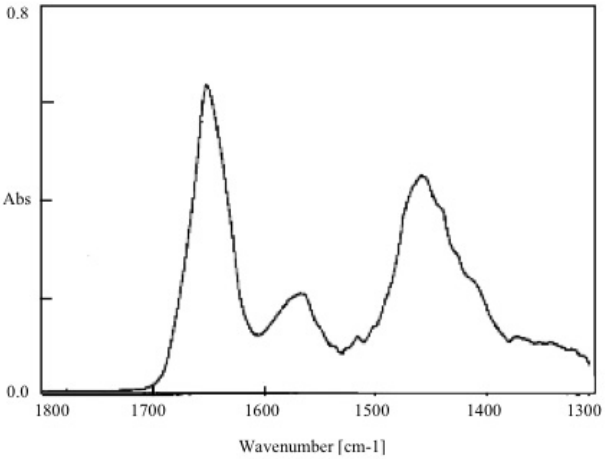

Figure 5 Cytochrome C

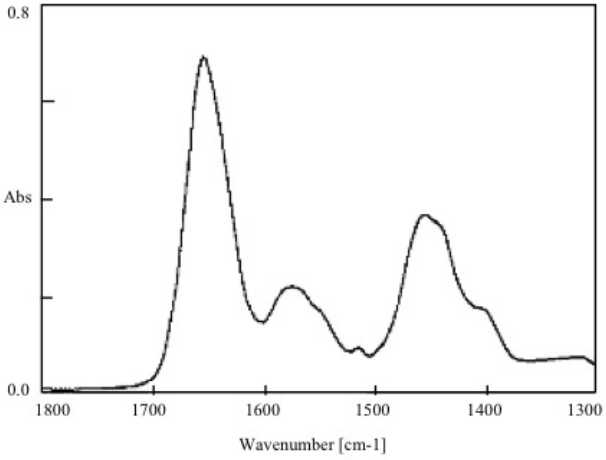

Figure 6 Bovine serum albumin

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