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Application News

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Spectrophotometric Analysis

Evaluation of Amyloid-β Aggregation by Fourier Transform Infrared Spectrophotometer (FTIR)

Amyloid-β is a peptide that consists of approximately 40 amino acid residues. Amyloid fibrils (fibrous aggregates), which occur as a result of the formation of parallel β sheets by intermolecular association of amyloid-β, are the main component of the senile plaques (amyloid plaques) seen in the brains of Alzheimer's disease patients. Moreover, the formation mechanism and structure of amyloid fibrils have attracted great interest, as amyloid fibrils are also implicated in other neurodegenerative diseases, including Parkinson's disease [1].

With FTIR, it is possible to evaluate the aggregation of amyloid-β by analyzing the amide I band around 1,650 cm^{-1}, which originates from stretching vibrations of the C=O group of the peptide bond. Fig. 1 shows the peptide bond structure. The secondary structures (α-helix, β-sheet, β-turn, random coil, and other local 3-dimensional conformational features) of the amyloid-β peptide can be obtained by curve fitting (peak splitting). Curve fitting is method in which the waveforms of each absorption band are expressed by an approximate curve such as a Lorentzian curve or Gaussian curve, and the peak information (position, intensity, full width at half maximum) of the approximate curve of each absorption band is optimized so as to minimize the difference between the calculated spectrum and the measured spectrum [2]. This article introduces the results of an evaluation of amyloid-β aggregation.

R. Fuji, K. Maruyama, S. Iwasaki

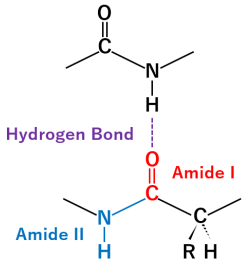

Fig. 1 Structural Diagram of Peptide Bonds

Instruments and Measurement Conditions

In these measurements, a Shimadzu IRTracer™-100 Fourier transform infrared spectrophotometer and a MicromATR™ attenuated total reflectance (ATR) measurement accessory (Czitek, LLC.) were used. The ATR method makes it possible to obtain a spectrum simply by placing the sample in close contact with the crystal, and cleaning after measurements is also simple in comparison with the transmission method. Moreover, the 9 reflection ATR crystal of the MicromATR accessory enables measurement with higher sensitivity than with the widely-used single reflection devices. Here, it may be noted that the optical system was purged with dry air because the peaks of the amide I band and water vapor overlap.

Table 1 Measurement Conditions

Instruments	: IRTracer-100
	: MicromATR™ (9 reflection ATR crystal)
Resolution	: 4 cm^{-1}
Accumulation	: 100 times
Zero filling	: 4 ×
Apodization function	: Sqr-Triangle
Detector	: DLATGS

Experimental Procedure

Samples were prepared by dissolving amyloid-β (human, 1-40) to approximately 10 μM with ultrapure water, and ATR measurements were carried out after allowing the samples to stand at 25 °C for 0.5, 1, 2, 3, 24, 48, or 120 h. In this experiment, 60 μL of the amyloid-β solution was dripped (30 μL × 2 times) on the ATR crystal, the water content was completely removed with dry air, and the exsiccated amyloid-β on the crystal was then measured.

Although the 9 reflection ATR crystal is intended for use exclusively with liquids, it is possible to measure samples in solid form after exsiccation of the liquid on the crystal as described above. After the measurement, the crystal was cleaned by wiping off the sample on the crystal with water without applying force.

Curve Fitting Procedure

The secondary structures of the amyloid-β were identified by the following procedure.

- From 10 to 14 negative peaks were detected in the range of 1,700 cm^{-1} to 1,600 cm^{-1} in the second derivative spectrum of amyloid-β. These peaks were used as the designated wavenumbers when conducting curve fitting.
- In this analysis, curve fitting was done by using the Lorentzian function. The conditions were adjusted to obtain the best match of the infrared spectrum of amyloid-β before curve fitting and the synthesized spectrum of the peaks separated by curve fitting in the range of 1,750 cm^{-1} to 1,575 cm^{-1}.
- The secondary structure types were assigned to the separated peaks based on Reference [3]. The fraction of each type of secondary structure was calculated by the ratio to the total area of each curve.

Measurement Results

Fig. 2 shows the amide I band in the infrared spectrum of amyloid-β. The spectra in Fig. 2 have been normalized so that the maximum peak intensity of the amide I band shows absorbance of 0.03. The peak at 1,626 cm^{-1} (0.5 h) shifted to 1,630 cm^{-1} (120 h) with time.

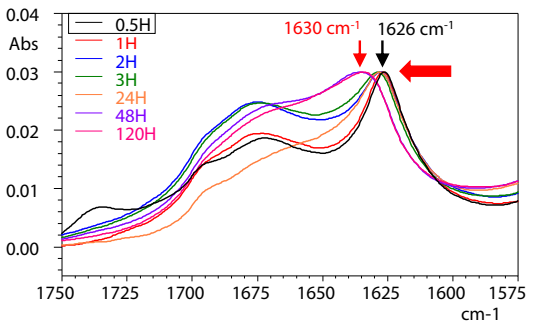

Fig. 2 Amide I Band in Infrared Spectrum

Fig. 3 shows the second derivative spectrum of amyloid-β. The second derivative spectrum revealed that the intensities of the peaks at 1,696 cm^{-1} and 1,626 cm^{-1} decrease and the peak at 1,626 cm^{-1} shifts to 1,630 cm^{-1}. The peaks at 1,696 cm^{-1} and 1,626 cm^{-1} are a distinctive feature of antiparallel β sheets, whereas the peak at 1,630 cm^{-1} is caused by parallel β sheets [5]. Fig. 4 shows the β sheet structure.

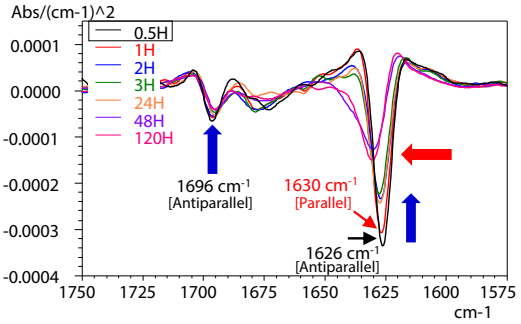

Fig. 3 Second Derivative Spectrum

Next, the secondary structures were identified by curve fitting of the amide I band. Fig. 5 shows the infrared spectra after 0.5 h and 120 h and the results of curve fitting. The peaks in the ranges of 1,700 cm^{-1} to 1,690 cm^{-1} (high wavenumber region) and 1,645 cm^{-1} to 1,615 cm^{-1} (low wavenumber region) were assigned to the β sheet [3]. Table 2 shows the sums of the peak areas and the ratios of the high and low wavenumber regions of the β sheets. The ratio after 0.5 h was 0.2836, suggesting a structure with a large fraction of antiparallel β sheets. After 120 h, the ratio decreased to 0.1860, clarifying the fact that conversion from the antiparallel β sheet structure to the parallel β sheet structure occurs over time, and the number of amyloid fibrils caused by formation of parallel β sheets also increases. This is the same result as the second derivative spectrum described above.

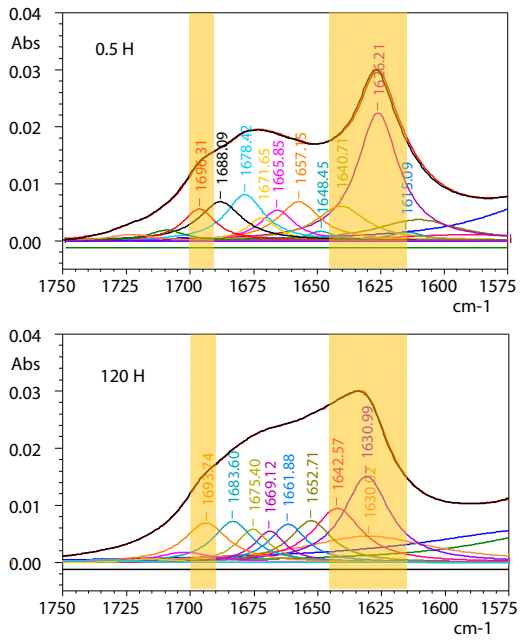

Fig. 5 Infrared Spectra and Results of Curve Fitting

Table 2 Sum of Peak Areas and Ratios of β Sheets

		0.5 h	120 h
Sum of peak areas	High wavenumber: 1,700 cm^{-1} - 1,690 cm^{-1}	0.2371	0.2115
	Lower wavenumber: 1,645 cm^{-1} - 1,615 cm^{-1}	0.8359	1.1372
Ratio (high wavenumber/low wavenumber)		0.2836	0.1860

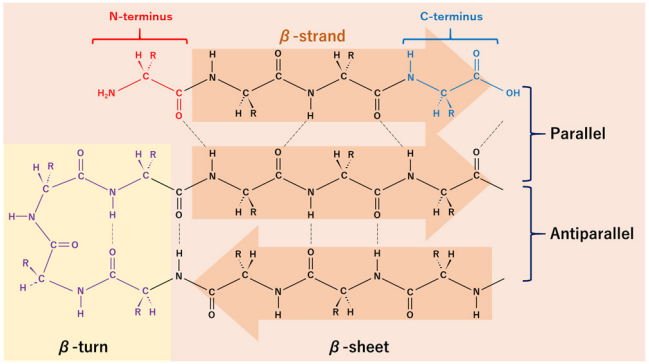

Fig. 4 Structure of β Sheet

Conclusion

Amyloid-β aggregation was evaluated by using FTIR. The results of an analysis of the amide I bands in the infrared spectrum and second derivative spectrum suggested that a time-dependent change (conversion from antiparallel β sheet structure to parallel β sheet structure) occurs in the secondary structure accompanying aggregation of amyloid-β.

<References>
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