Synchrotron Radiation Infrared Microscopy Analysis of Amyloid Fibrils in Alzheimer's Disease Model Mouse Brain Tissue

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Abstract

A mid-infrared free electron laser (FEL) is a linearly polarized, high-peak powered pulse laser with tunable wavelength within mid-infrared absorption region. We previously found that the FELs tuned to amide bands (I, C=O stretching vibration; II, N-H bending vibration; III, C-N stretching vibration) could convert amyloid fibrils as well as keratin aggregate into their non-aggregated forms. In this study, we irradiated the FEL at 6.17 μm to mouse brain tissue section including amyloid fibrils of Alzheimer's disease, and analyzed the secondary structure of proteins in the brain section by using BL15 infrared microscopy beam line. The IR spectra obtained by the analysis showed that β-sheet content in the transgenic mouse brain section was reduced to almost the same level with that of wild type mouse brain section after the FEL irradiation. This result indicates that amyloid fibrils in the biological tissue can be dissociated by the FEL irradiation.

1. Introduction

A mid-infrared free-electron laser (FEL) is generated by an interaction of synchrotron radiation with an electron beam. The features of the FEL are 1) a specific pulse profile with complete linear polarization, 2) tunable frequency within infrared region, and 3) high photon density [1]. These characteristics encourage us to apply the FEL for spectroscopic studies and thermodynamic analyses of bio- and material molecules, and surgical ablation of biological tissues [2, 3]. We have recently found that the FEL tuned to amide bands (amide I: C=O stretching, amide II: N-H bending, and amide III: C-N stretching vibration modes) can dissociate not only amyloid fibrils of lysozyme and insulin peptide but also keratin aggregate into their monomer structures [4, 5, 6]. Although the FEL can be expected to be used for reducing pathogenic protein aggregates from the biological tissues, it is necessary to examine the efficacy of the FEL in complex biological tissues in order to apply the FEL to the therapy of protein aggregation diseases.

In this study, we tested if the FEL would dissociate amyloid fibrils in mouse brain tissue section, and found that β-sheet contents in the brain section could be reduced by the irradiation at the amide I band.

2. Experimental

FEL irradiation method

The experimental setup was described in details in previous studies [4, 5, 6]. The pulse energy per macro-pulse used in this study was in the range of 8.0–10.0 mJ, the spot size of beam line was ca. 0.5 cm in diameter, and the power densities of the FEL were estimated to be 35 – 45 mJ/cm^2. The FELs with various wavelengths were irradiated into the mouse brain section on the stainless steel base at 37 °C for 1 h. After the irradiation was completed, the sample was air-dried and subjected to the spectroscopy analysis as described below.

SR-IRM analysis

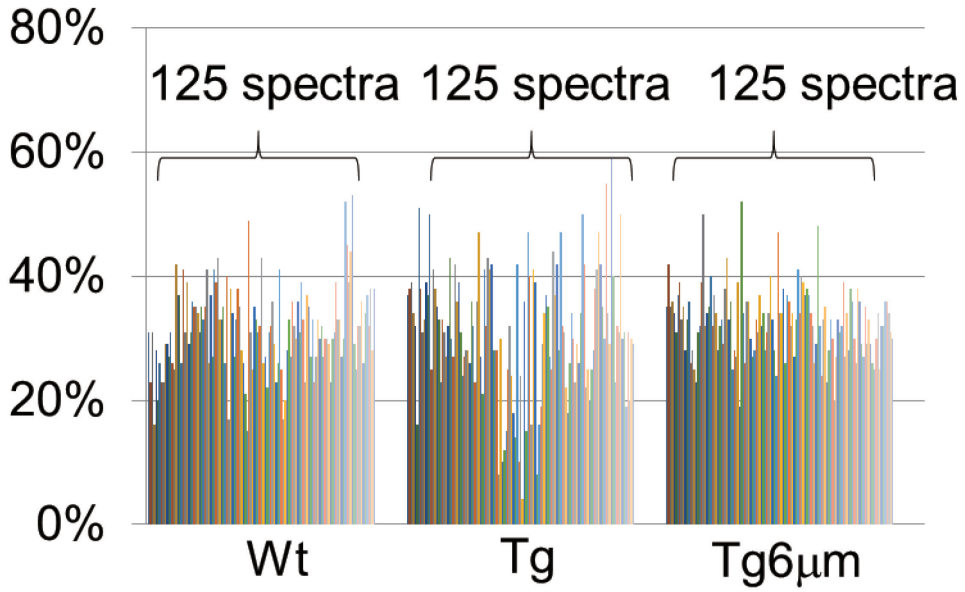
The synchrotron-radiation infrared microscopy (SR-IRM) analysis was performed using the IR micro-spectroscopy beam line (SRMS, BL-15) at the SR center of Ritsumeikan University (Kusatsu, Shiga, Japan) [7]. The beam line is equipped with Nicolet 6700 and Continuμm XL IR microscopes (Thermo Fisher Scientific Inc.). Measurements were

performed in reflection mode with a 32× Cassegrain lens and a 20 μm × 20 μm aperture. Total 125 spectra were collected in the mid-IR range of 700–4000 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans from five regions by lattice measurement (x-axis: 100 μm, y-axis: 100 μm at each region). Smoothing and normalization of spectra were performed on the region containing amide bands (1000-2000 cm^{-1}) by using Spectra Manager software Ver. 2 (Jasco International Co., Ltd., Tokyo, Japan). Contents of main conformations in peptide, α-helix, β-sheet, β-turn, and other conformation could be calculated by de-convolution of amide I band (1600-1700 cm^{-1}) using the protein analysis software (IR-SSE, JASCO Co., Ltd.).

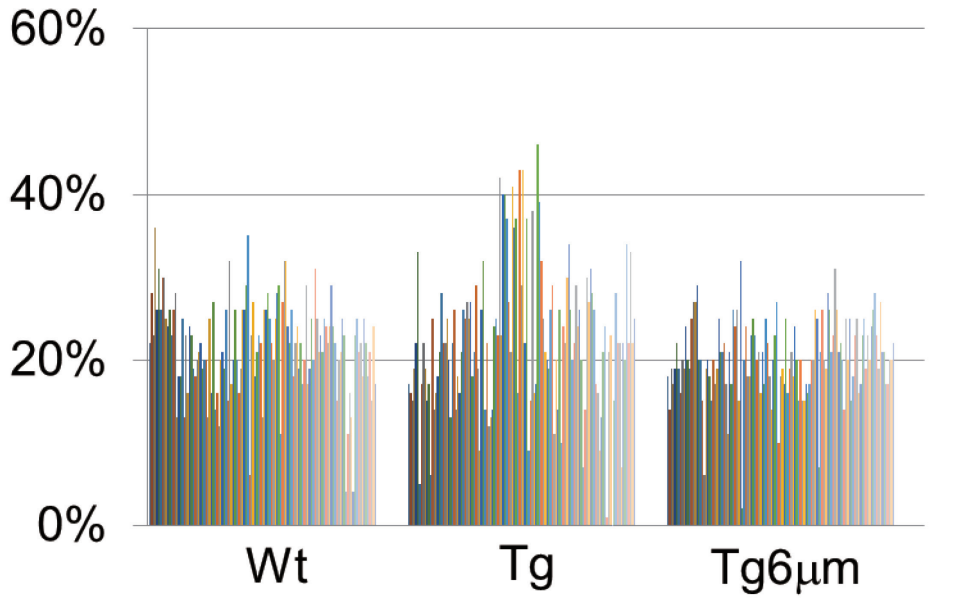
3. Results and Discussion

SR-IRM improves the spatial resolution with a high signal-to-noise (S/N) ratio compared to IRM using a thermal radiation beam because high-power radiation can be delivered to a limited area in a small sample of several micrometers section [8]. Secondary structure contents of the brain section were shown in Figure 1. At each brain section, total 125 spectra ware acquired. In Fig. 1(A), α-helix content of the transgenic mouse brain section (Tg) was slightly low compared to that of the wild type section (Wt). On the contrary, that was increased to almost the same level with that of the wild type section after the FEL irradiation at 6.17 μm (Tg6μm). In Fig. 1(B), β-sheet content (around 40%) in “Tg” was found to be apparently higher than that of “Wt”. This indicates that β-sheet-rich amyloid fibrils were accumulated in the transgenic mouse brain. On the contrary, that was decreased as low as that of the wild type after the FEL irradiation (Tg6μm). From the results of the β-sheet reduction and α-helix increase after the FEL irradiation, it may be apparent that amyloid fibrils even in the brain section could be dissociated by the irradiation. Since β-turn and other conformers were almost the same levels in all brain sections, it can be estimated that the FEL irradiation could little affect other structures of proteins than amyloid fibril structure.

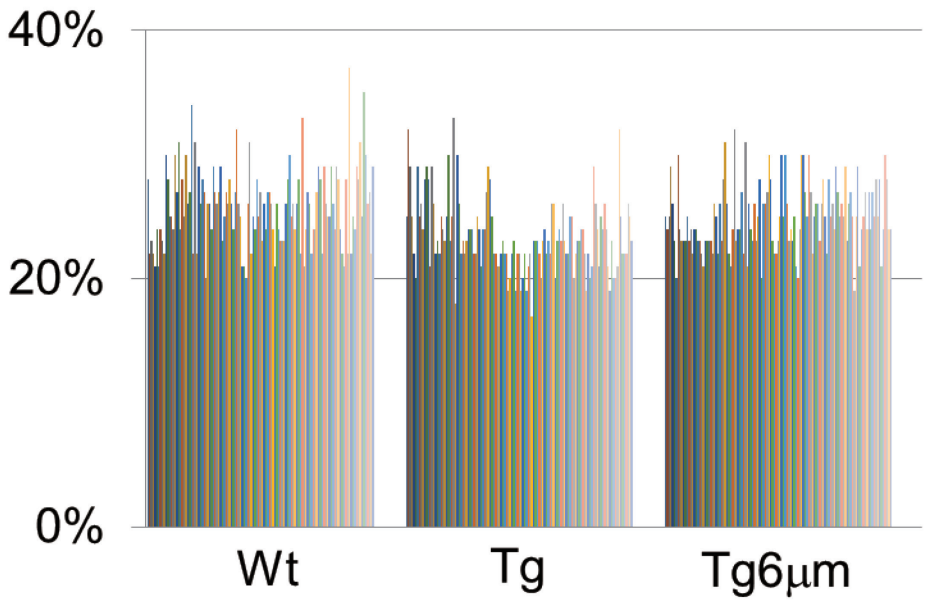
(A)


80%
60%
40%
20%
0%
125 spectra
125 spectra
125 spectra
Wt
Tg
Tg6μm

(B)


60%
40%
20%
0%
Wt
Tg
Tg6μm

(C)



(D)

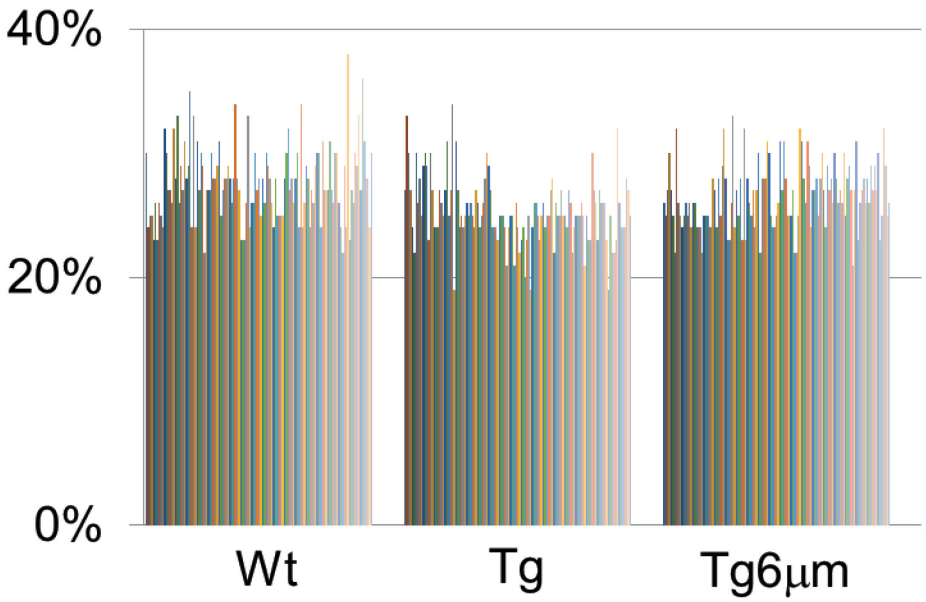


Figure 1 Secondary structure contents calculated based on the SR-IRM spectra. "Wt" indicates a wild type mouse brain section, "Tg" indicates a transgenic mouse brain section, and "Tg6μm" indicates the transgenic mouse brain section after the FEL irradiation at 6.17 μm, respectively. (A) α-helix, (B) β-sheet, (C) β-turn, and (D) other conformation

4. Conclusions

Dissociation of amyloid fibrils to the non-fibril states in the mouse brain section could be driven by mid-IR FEL irradiation tuned to amide I band similarly with the amyloid fibrils without biological tissues. The mid-IR FEL is expected to be used for reducing the amyloid fibrils from Alzheimer's disease model mouse brain.

Acknowledgement

We thank staffs at FEL-TUS for operating the FEL instrument. This work was supported in part by the Open Advanced Research Facilities Initiative and Photon Beam Platform Project of the Ministry of Education, Culture, Sport, Science and Technology, Japan.

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